AD_____

Award Number: DAMD17-00-1-0431

TITLE: Breast Cancer Susceptibility Genes in High Risk Women

PRINCIPAL INVESTIGATOR: Ann S. Hamilton, Ph.D.

CONTRACTING ORGANIZATION: University of Southern California
Los Angeles, California 90033

REPORT DATE: July 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2001	Annual (1 Jul 00 - 30 May 01)

4. TITLE AND SUBTITLE
Breast Cancer Susceptibility Genes in High Risk Women

5. FUNDING NUMBERS
DAMD17-00-1-0431

6. AUTHOR(S)
Ann S. Hamilton, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Southern California
Los Angeles, California 90033

E-Mail: ahamilt@usc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited.

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*
A positive family history, present in about 30% of breast cancer cases, has been shown to double a woman's risk of breast cancer. The genetic factors responsible are largely unknown, although the autosomal dominant, relatively high penetrant genes BRCA1/2 may account for 3%. It has been hypothesized that susceptibility genes of lower penetrance may also affect breast cancer risk, and a likely group of such genes are those that regulate the production, intracellular transport, and metabolism of estrogen. Previous studies of these susceptibility genes have not compared women with high familial risk to those with lower risk. We are studying identical twins with differing genetic risks (i.e. concordant for breast cancer pairs vs. discordant pairs) as well as unaffected controls and are comparing the frequency of polymorphisms in selected genes related to estrogen and carcinogen metabolism in each of these groups. In the estrogen metabolism pathway, polymorphisms related to the CYP17, CYP19, COMT, and HSD17B1 genes are being studied as are polymorphisms in the GSTM1, P1 and CYP1A1 genes in the carcinogen metabolism pathway. The final sample size will include 200 women in each group. We have developed the protocols and procedures for the study and currently have tissue from 111 concordant and 148 discordant pairs and buccal smears from 3 control women. Informed consents (specifically for this study) have been obtained from 26 concordant, 54 discordant, and 3 control women. Laboratory analyses of the CYP17 gene are underway.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer, CYP17, CYP19, COMT, CYP1A1, HSD17B1, GSTM1, Twins, estrogen metabolism, carcinogen metabolism, genetics	21
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

4) INTRODUCTION

A positive family history, present in about 30% of breast cancer cases, has been shown to double a woman's risk of breast cancer(1), and this is true for postmenopausal as well as the premenopausal cases, among which the autosomal dominant, relatively high penetrant genes BRCA1 and BRCA2 are most prominent(2). It has been hypothesized that susceptibility genes of lower penetrance are more prevalent than among the latter, and a likely group of such genes are those that regulate the production, intracellular transport, and metabolism of estrogen (3), the common factor underlying most known predictors of breast cancer risk (4) (5) (6). Recent reviews have identified several candidate genes (7) (8) (9). We have chosen to focus on those genes related to estrogen metabolism and carcinogen metabolism.

In the estrogen metabolism pathway, four genetic polymorphisms have been described related to the CYP17 gene, the CYP19 gene, the COMT gene, and the HSD17B1 (or also called the EDH17B2) gene. For example, a polymorphism (called A2) on the CYP17 gene has recently been linked to higher endogenous estrogen levels and an earlier age at menarche (10). The same polymorphism was linked to increased risk of aggressive breast cancer, although one attempt to confirm this finding was unsuccessful(11). Genes related to carcinogen metabolism which have been linked to breast cancer risk include GSTM1 and P1 and CYP1A1. These studies, however, have not been conducted with women known to be at high familial risk, where the prevalence of the polymorphism may be expected to be higher, if it is associated with the development of breast cancer. This study proposes to take advantage of a unique subset of very high risk women in whom cumulative exposure to endogenous estrogen may play an especially important role in breast cancer etiology.

The identification of families to study these inherited genetic factors is more difficult because of the anticipated lower penetrance of the candidate genes and occurrence of more sporadic cases, especially among older women. The International Twin Study includes both breast cancer concordant and discordant identical twin pairs. The concordant MZ twin pairs represent families with a very high familial risk of breast cancer, while the MZ discordant twins are likely to represent non-heritable cancer. We plan to obtain DNA from subsets of these pairs as well as from control women without breast cancer (and without a family history of breast cancer) and to test for the genetic polymorphisms specified to determine if any are differentially associated with cases from twins with a high likelihood of heritable breast cancer (i.e. those from identical concordant pairs). This study should provide important clues regarding other genetic factors that may be associated with breast cancer etiology. Initial work on the project and the CYP17 laboratory work is funded under a grant from the California Breast Cancer Research Project (CA-BCRP).

5) BODY

The Human Subjects approval from the DOD and the USC IRB was obtained. The approved consent forms are included as Appendix 1. A detailed revised human subjects protocol was approved by the DOD. This process took approximately 5 months.

Technical Objectives and Work Accomplished in year 1:

Task 1: To complete follow-up of female identical twin pairs with breast cancer (Months 1-18)

1. *Continue follow-up begun under CA-BCRP grant*
2. *Hire Programmer, set up tracking database*
3. *Continue to mail follow-up forms with return envelope to last known address of twins. Enter data from responses.*
4. *Submit nonrespondent names to National Death Index.*
5. *Submit names of nonrespondent twins not known to be deceased to TRW/ Experian to obtain updated addresses. Resend follow-up forms.*
6. *Continue follow-up by phone calls, internet searches, and contact with relatives.*

A data file was created from the International Twin Registry that selected all of the identical female twin pairs in which one or both members had been diagnosed with breast cancer. In total there are 1,491 identical pairs in this database and 1,199 of them were initially classified as discordant pairs, 263 as concordant, and 29 of uncertain concordance. A follow-up form was sent to all living members of all of the discordant pairs, and new breast cancers have been reported in the previously healthy twin of 62 of these pairs. Thus as a result of this information, there are now 338 concordant pairs and 1,153 discordant pairs.

A total of 1,883 follow-up forms have been mailed to living twins in these pairs, and 1,029 have been returned completed. 260 were returned by the post office and 478 are currently not returned by either the twin or the post office. Tracing efforts have been implemented to locate the nonrespondents. At the present time 852 twins have been traced using Experian and matches were found for 632 of them (74%). The remaining 220 without a match will be further traced by linkage with the National Death Index. Of the twins with matches, a new address was found for 270 and the same address was found for 362. Follow-up on the nonrespondents will continue.

A tracking database has been developed using Microsoft Access and the main staff person on the project (Mary Lo) is doing the programming work required. A file to the NDI is currently being developed, will be submitted within the next month. (This component is funded under the CA-BCRP grant).

Task 2: Identify new breast cancers and obtain medical record documentation and tissue blocks. (Months 6-20)

1. *When new breast cancer is identified, obtain medical consent form from twin or next of kin, and request records and tissue blocks from hospital*
2. *Follow-up requests with hospitals*

The goal of the study is to obtain genomic DNA from at least one member of 200 of the concordant pairs, from the case in 200 of the discordant pairs, and from 200 control women without a personal or family history of breast cancer. From a previous study tissue, blocks have been obtained from some of the breast cancer pairs (concordant and discordant). As a result of the follow-up effort, we have identified 62 previously discordant pairs in whom the unaffected

member has developed breast cancer. Thus the number of concordant and discordant pairs has been adjusted to reflect the current status.

To participate in the study, the eligible participants are sent a letter describing the study along with the informed consent documents. Our study manager then calls the twin to go over the informed consent with her over the telephone. Then if she agrees to participate and donate the required tissue to the study, she then signs the informed consent form and mails it back to us. (These procedures are detailed in the approved human subjects protocol).

As of this time (7/27/01) the current numbers of MZ twins (and controls) in each subset with tissue and signed consent forms is the following:

	Concordant	Discordant	Controls
Number identified	265*	976*	45**
Buccal kit sent			20
Tissue available	111	148	
Buccal smear received			3
Additional cases who could be sent buccal smear kit	154	828	
DOD consent signed and tissue/buccal smear available	26	54	3
Goal	200	200	200

*at least one alive (concordant pairs) or case alive (discordant pairs)
**control selection process has just begun

We are continuing the process of obtaining the DOD consent form from the concordant and discordant cases with tumor tissue available. In order to reach our sample size goal of 200 in each category we will select from the 154 concordant pairs (with at least one twin alive) and the 828 discordant pairs (case alive).

Task 3: Obtain buccal smears from living member of case pairs when blocks not available (Months 1-20)

1. *If tissue blocks are no longer available from either member of the case pairs and there is a living twin, send letter to obtain buccal smear.*
2. *Send buccal smear kit and return mailing supplies and postage to these individuals.*

The procedures for obtaining buccal smears have been developed and kits have been assembled for this purpose. We are using Epicentre Technologies Master Amp Buccal Swab Brush. Two brushes are being sent to the selected cases (and controls) and they are asked to use one for each cheek. Once the swabs are returned to us they are being kept frozen until the laboratory analyses are done. We have not yet sent these kits out to cases; however we have sent them to 20 controls to date and have received 3 back.

Task 3: Identify 200 control women and obtain buccal smear and risk factor questionnaire from each of them
(Months 1-20)

1. *Contact case pairs to obtain listing of unrelated breast cancer free potential control women selected from sisters-in-laws and friends.*
2. *Randomly select a women from this list and mail introductory letter.*
3. *Obtain buccal smear and risk factor questionnaire from each control woman through the mail.*

We have developed the protocol for selecting controls. The case is asked to list sisters-in-law that are close to their age (and then friends if no sisters-in-law are available). Once we have received permission from the case to contact a sister-in-law, our study manager Mary Lo will call the control first to determine if she is eligible, as follows:

Hello_____, my name is Mary from the Twin Study and we received your name from _____. Before we can include you in the study I need to ask you a few preliminary questions. This information is kept confidential and your participation is voluntary:

1) Have you ever been diagnosed with cancer? Yes_____, No_____
   If Yes: What type?_____
   (If other than basal cell skin cancer, then say, Thank you , but you are not eligible for the study, and end interview—find new control)
2) Has your mother or any sister or daughter been diagnosed with breast cancer?
   Yes _____; No_____
   If Yes: Thank you , but you are not eligible for the study, and end interview—find new control)

If No to both questions, then say, 'You would be eligible for our study'. I will send you all of the information about it including an informed consent form and then you can decide if you would like to participate. (If they ask: Briefly your involvement would include answering a questionnaire and providing a DNA sample from cells from inside your mouth that would be obtained by swishing some mouthwash in your mouth and spitting it into a tube).

If everything is OK, then Mary will verify address and send the materials to the control woman.

1) Letter with consent form, buccal smear kit, questionnaire, separate return envelopes for consent form, questionnaire, and buccal smear kit.
2) Mary will call them and go over the consent form as she does with the cases.

To date we have identified 45 controls and have sent the kits to 20 of them, and three have been returned. In the process of going over the DOD informed consent with the cases and controls we have had some problems with the DOD required statements regarding the donation of the sample and 1 twin refused to participate as a result.

Task 4: Laboratory analysis of DNA from tissue and buccal smears to identify polymorphisms in the specified breast susceptibility candidate genes
(Months 1-24)

1. *Finish CYP-17 analysis at Dr. Dubeau's Laboratory.*
2. *Extract additional DNA as necessary for the additional genetic tests.*
3. *Do additional tests for CYP19, COMT, HSD17B1, GSTM1, GSTP1, and CYP1A1.*
4. *Receive results and enter data into database.*
5. *Store tissue for future genetic studies.*

We have developed a system for removing original study id numbers from the slides and have de-linked them from personal identifiers. We have currently sent tissue from 136 cases (59 from concordant and 77 from discordant pairs) to Dr. Dubeau's laboratory for the CYP17 analysis. The results are expected by late August. The molecular biology core laboratory is equipped with real-time PCR apparatus (Taqman 7700, Perkin Elmer Corporation). This apparatus is more sensitive than most conventional PCR-based methods for the detection of polymorphic alleles in genotyping studies. Given that our study relies on archival tissue samples, which are more difficult to amplify by PCR, this increased sensitivity is important. The most significant impact of the Taqman apparatus, however, will be in greatly increasing turn around time. Genotyping studies based on classical approaches such as those initially mentioned in our proposal involve a PCR reaction followed by a restriction endonuclease digestion of the PCR product, which subsequently needs to be electrophoresed on agarose gels. Finally, the gels need to be interpreted, introducing an element of subjectivity, and the results need to be entered manually in a database. These multiple steps span over several days. The Taqman instrument allows us to complete all the analysis in a single step. It eliminates the need for restriction enzyme digestions, electrophoresis, and subjective interpretation. The samples are loaded on the Taqman apparatus that completes the analysis, scores each sample, and automatically enters the results in a database format within approximately 2 hours.

Task 4 Data analysis (Months 18-32)
1. *Link data on genetic factors to other information from twins and controls including risk factor information and other tumor related information when available (e.g. ER positivity)*
2. *Complete analyses of data to determine relationship of the specified polymorphisms to breast cancer susceptibility.*
3. *Submit papers and reports.*

Work on this phase of the project has not started yet.

6) Key Research Accomplishments

a. Protocols for all aspects of the study have been developed.

b. Follow-up has largely been completed and 62 new concordant pairs have been identified.
c. The control selection has been started and is working well.
d. Initial samples have been sent to the laboratory for CYP17 analyses.

7) Reportable Outcomes

a. 62 new concordant pairs have been identified from the follow-up effort.
b. We have completed DNA sample collection and obtained signed informed consents from 26 concordant cases , 54 discordant cases and 3 controls. The goal is to obtain 200 in each group by the completion of the study.

8) Conclusions

Human subjects protocols and approvals from both the DOD and the USC IRB delayed the implementation of the study; however now all these requirements have been met and protocols for control selection and buccal smear collection have been developed. It is anticipated that the next year will result in the continued recruitment of study subjects at a much faster rate.

9) REFERENCES

1. Byrne C, Brinton LA, Haile RW, Schairer C. Heterogeneity of the effect of family history on breast cancer risk. Epidemiology 1991;2:276-84.
2. Castilla LH, Couch FJ, Erdos MR, et al. Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. Nature Genetics 1994;8:387-91.
3. Feigelson HS, Ross RK, Yu MC, Coetzee GA, Reichardt JK, Henderson BE. Genetic susceptibility to cancer from exogenous and endogenous exposures. Journal of Cellular Biochemistry - Supplement 1996;25:15-22.
4. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiologic Reviews 1993;15:36-47.
5. Bernstein L, Ross RK. Endogenous hormones and breast cancer risk. Epidemiologic Reviews 1993;15:48-65.
6. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiologic Reviews 1993;15:17-35.
7. Rebbeck TR. Inherited genetic predisposition in breast cancer. A population-based perspective. Cancer 1999;86:2493-501.
8. Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiology, Biomarkers & Prevention 1999;8:843-54.
9. Coughlin SS, Piper M. Genetic polymorphisms and risk of breast cancer. Cancer Epidemiology, Biomarkers & Prevention 1999;8:1023-32.
10. Feigelson HS, Coetzee GA, Kolonel LN, Ross RK, Henderson BE. A polymorphism in the CYP17 gene increases the risk of breast cancer. Cancer Research 1997;57:1063-5.
11. Dunning AM, Healey CS, Pharoah PD, et al. No association between a polymorphism in the steroid metabolism gene CYP17 and risk of breast cancer. British Journal of Cancer 1998;77:2045-7.

12. Ford D, Easton DF, Peto J. Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. American Journal of Human Genetics 1995;57:1457-62.
13. Rowell S, Newman B, Boyd J, King MC. Inherited predisposition to breast and ovarian cancer [editorial; comment]. American Journal of Human Genetics 1994;55:861-5.
14. Martucci CP, Fishman J. P450 enzymes of estrogen metabolism. Pharmacology & Therapeutics 1993;57:237-57.
15. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17alpha gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Research 1998;58:585-7.
16. Siegelmann-Danieli N, Buetow KH. Constitutional genetic variation at the human aromatase gene (Cyp19) and breast cancer risk. British Journal of Cancer 1999;79:456-63.
17. Kristensen VN, Andersen TI, Lindblom A, Erikstein B, Magnus P, Borresen-Dale AL. A rare CYP19 (aromatase) variant may increase the risk of breast cancer. Pharmacogenetics 1998;8:43-8.
18. Huang CS, Chern HD, Chang KJ, Cheng CW, Hsu SM, Shen CY. Breast cancer risk associated with genotype polymorphism of the estrogen-metabolizing genes CYP17, CYP1A1, and COMT: a multigenic study on cancer susceptibility. Cancer Research 1999;59:4870-5.
19. Thompson PA, Shields PG, Freudenheim JL, et al. Genetic polymorphisms in catechol-O-methyltransferase, menopausal status, and breast cancer risk. Cancer Research 1998;58:2107-10.
20. Millikan RC, Pittman GS, Tse CK, et al. Catechol-O-methyltransferase and breast cancer risk. Carcinogenesis 1998;19:1943-7.
21. Pasqualini JR. Role, control and expression of estrone sulfatase and 17 beta-hydroxysteroid dehydrogenase activities in human breast cancer. Zentralblatt fur Gynakologie 1997;119:48-53.
22. Miettinen MM, Mustonen MV, Poutanen MH, Isomaa VV, Vihko RK. Human 17 beta-hydroxysteroid dehydrogenase type 1 and type 2 isoenzymes have opposite activities in cultured cells and characteristic cell- and tissue-specific expression. Biochemical Journal 1996;314:839-45.
23. Peltoketo H, Vihko P, Vihko R. Regulation of estrogen action: role of 17 beta-hydroxysteroid dehydrogenases. Vitamins & Hormones 1999;55:353-98.
24. Henderson B, Feigelson HS. Hormonal carcinogenesis. Carcinogenesis 2000;21:427-433.
25. Mannermaa A, Peltoketo H, Winqvist R, et al. Human familial and sporadic breast cancer: analysis of the coding regions of the 17 beta-hydroxysteroid dehydrogenase 2 gene (EDH17B2) using a single-strand conformation polymorphism assay. Human Genetics 1994;93:319-24.
26. Peltoketo H, Nokelainen P, Piao YS, Vihko R, Vihko P. Two 17beta-hydroxysteroid dehydrogenases (17HSDs) of estradiol biosynthesis: 17HSD type 1 and type 7. Journal of Steroid Biochemistry & Molecular Biology 1999;69:431-9.
27. Peltoketo H, Piao Y, Mannermaa A, et al. A point mutation in the putative TATA box, detected in nondiseased individuals and patients with hereditary breast cancer, decreases

promoter activity of the 17 beta-hydroxysteroid dehydrogenase type 1 gene 2 (EDH17B2) in vitro. Genomics 1994;23:250-2.

28. Charrier J, Maugard CM, Le Mevel B, Bignon YJ. Allelotype influence at glutathione S-transferase M1 locus on breast cancer susceptibility. British Journal of Cancer 1999;79:346-53.
29. Helzlsouer KJ, Selmin O, Huang HY, et al. Association between glutathione S-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer [see comments]. Journal of the National Cancer Institute 1998;90:512-8.
30. Huang CS, Shen CY, Chang KJ, Hsu SM, Chern HD. Cytochrome P4501A1 polymorphism as a susceptibility factor for breast cancer in postmenopausal Chinese women in Taiwan. British Journal of Cancer 1999;80:1838-43.
31. Taioli E, Bradlow HL, Garbers SV, et al. Role of estradiol metabolism and CYP1A1 polymorphisms in breast cancer risk. Cancer Detection & Prevention 1999;23:232-7.
32. Deapen D, Escalante A, Weinrib L, et al. A revised estimate of twin concordance in systemic lupus erythematosus [see comments]. Arthritis & Rheumatism 1992;35:311-8.

10) APPENDICES

Approved informed consent documents

Proposal #**001034**
Review Category: **C**

INSTITUTIONAL REVIEW BOARD
HEALTH RESEARCH ASSOCIATION
AND
INSTITUTIONAL REVIEW BOARD
UNIVERSITY OF SOUTHERN CALIFORNIA
SCHOOL OF MEDICINE

Date: 05/25/01
To: **Ann S Hamilton, Ph.D.**
Assistant Professor
Preventive Medicine
Norris Cancer Center, #3427
HEALTH SCIENCES CAMPUS

From: **Vice Chair, IRB**
Robert Larsen, M.D.
Trailer #25, Unit I
1200 N. State Street
Los Angeles, CA 90033
(323) 223-2340/2349

TITLE OF PROPOSAL:
BREAST CANCER SUSCEPTIBILITY GENES IN HIGH RISK WOMEN (DODBCRP)

Action Date: **5/24/01** Action Taken: **Approved**
Committee: Vice Chair, IRB
Note:

Your correspondence dated 5/9/01 and attachments were reviewed by Dr. Larsen on 5/24/01. The proposed changes qualify for expedited review according to 45CFR46.110 (b) 2) minor changes in previously approved research during the period (of one year or less) for which approval is authorized. The proposed changes were APPROVED.

Revised informed consents #2 and #3, titled: "Description of Medical Research for Which Your Participation Is Requested", dated 5/8/01 were APPROVED.

Informed consent must be obtained by the investigator or person authorized to obtain informed consent from all research subjects or their legally authorized representatives. You must ensure that all project personnel involved in the process of consent/assent are trained properly and are fully aware of their responsibilities relative to the obtainment of informed consent/assent according to the IRB guidelines and applicable federal regulations.

The IRB office has stamped the approved informed consent form for use in this research project. It should be photocopied, as appropriate, onto the correct letterhead for the hospital or institute. You may not use this informed consent form document to consent new subjects after its expiration date. A photocopy of this IRB approved informed consent form document(s) bearing this stamp must be used for consenting and/or reconsenting the study subjects. The study subject must sign and date the informed consent document. The person obtaining informed consent must also sign the study consent form at the time consent is obtained. One copy of the informed consent should be given to the study subject, one copy placed in the hospital medical record, and the investigator should retain one copy. Jr/

USC Norris Comprehensive Cancer Center
Keck School of Medicine
Department of Preventive Medicine
1441 Eastlake Ave., Rm. 3427, MC9175
Los Angeles, CA 90089-9175

USC
SCHOOL OF
MEDICINE

2. Description of the Medical Research for which your participation is requested (Please sign and return one copy and keep the second copy for your records)

TITLE OF PROJECT: Breast Cancer Susceptibility Genes in High Risk Women

PRINCIPAL INVESTIGATOR: Ann Hamilton, Ph.D. (323)-865-0434

DEPARTMENT: Department of Preventive Medicine, Keck School of Medicine at the University of Southern California, 1441 Eastlake Ave, Los Angeles, CA 90089.

24-HOUR TELEPHONE NUMBER: Toll free number: 800-421-9631

PURPOSE OF THE STUDY: You are invited to participate in a research study of genetic factors that may be related to the development of breast cancer. You are invited to be a participant because you are a member of a twin pair in which one or both of you have been diagnosed with breast cancer.

The genes that we are studying include those that control the amount of estrogen produced in the body and those that control the removal of cancer causing substances from the body. (We will not be testing for BRCA1 and BRCA2, the rare genes that are known to increase risk of breast cancer). To test for these genes, we will use DNA that will be obtained from a sample of cells from the inside of your mouth that would be collected by swabbing the inside of your mouth with a soft brush.

PROCEDURES: We will send you a letter explaining this new study and ask you to collect a sample of cells from inside your mouth by swabbing the inside of your mouth with a soft brush and placing it in a plastic tube. You would then mail the tube to us. All of the materials and mailing supplies will be provided to you at no cost.

Laboratory analyses of the cell samples to determine the presence of genetic factors will be done with personal identifiers removed. Thus, it will not be possible to link results with specific individuals.

We may also ask you for your permission to contact a friend or relative without cancer to participate in the study, after you have discussed the study with them.

RISKS: There are no physical risks. For the sample collection in your mouth, there may be some slight discomfort. There is a small risk of loss of confidentiality, however all records are secured and kept confidential. While genetic testing will be done for this study, the results cannot be linked to a specific individual, thus risks related to genetic testing will not apply. For the genes being tested, the risk associated with development of breast cancer is unknown at this time.

BENEFITS: You may receive no direct benefit from your participation in this study. However, your participation may help us learn if certain genetic factors may be related to development of breast cancer.

ALTERNATIVES TO PARTICIPATION: An alternative would be not to participate in this study.

CONFIDENTIALITY STATEMENT: We will maintain the confidentiality of your medical records to the extent permitted by law. Data are kept in locked file cabinets and genetic information is not linked to personal identifying information. The information from this study may be published in scientific journals or presented at scientific meetings. Published results will only consist of numbers of persons arranged in categories. Representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as part of their responsibility to protect human subjects in research.

OFFER TO ANSWER QUESTIONS: You may contact the Principal Investigator at the number listed above if you have any questions about the study. If you have any questions regarding your rights as a research subject, you may contact the Institutional Review Board office (IRB) at 323-223-2340.

COMPENSATION: The availability and quality of your medical care will not be affected by your participation or refusal to participate. We can provide no compensation for your participation in this study.

VOLUNTARY PARTICIPATION AND WITHDRAWAL: Your participation in this research study is voluntary. Your decision whether or not to participate will not interfere with your right to health care or other services to which you are otherwise entitled. If you do decide to participate, you are free to withdraw your consent and discontinue participation at any time.

POTENTIAL FOR COMMERCIAL DEVELOPMENT RELATED TO RESEARCH AND DONATION OF CELL SAMPLES. The following paragraph is included at the request of the funding agency for the study, the Department of Defense. We feel that the possibility of a commercial application is remote.

During this study, you will be asked to provide cell samples from the inside of your mouth. These samples will be used for studying genes that may be related to breast cancer and may also be used for purposes that are currently unknown. There is a chance that the samples that you are donating under this study may be used in other research studies and may have some commercial value. Should your donated sample lead to the development of a commercial product, the Keck School of Medicine at the University of Southern California will own it and may take action to patent and license the product. The Keck School of Medicine at the University of Southern California does not intend to provide you with any compensation for your participation in this study nor for any future value that the sample you have given may be found to have.

	As a participant in this study called 'Breast Cancer Susceptibility Genes in High Risk Women', I voluntarily donate any and all cell samples to the Keck School of Medicine at the University of Southern California. I have read the above paragraph and agree that should my donated sample lead to the development of a commercial product, the Keck School of Medicine at the University of Southern California will own it and it is possible that it will be patented and licensed by the Keck School of Medicine at the University of Southern California . I will not receive any compensation for this.

Please write your initials in the box to indicate your agreement. Thank you.

INFORMATION ABOUT CELL SAMPLES COLLECTED AS PART OF THIS RESEARCH.

In the future it may be important to test these cells for newly discovered genetic factors that may be related to the development of breast cancer. We will be storing some of the cells for this purpose in the future. Please initial below if you give your consent for additional genetic testing to be done on these samples.

	I give consent for additional genetic testing related to breast cancer to be done in the future on my stored cell samples.

Please write your initials in the box to indicate your agreement. Thank you.

CONSENT PROCEDURE: Initial contact with participants in this study is made by a letter describing the study purpose and procedures. Participants are called and this document is discussed with them and they are asked to sign and return a copy of this document.

CALIFORNIA LAW REQUIRES THAT YOU MUST BE INFORMED ABOUT:

1. The nature and purpose of the study.
2. The procedures in the study and any drug or device to be used.
3. Discomforts and risks to be expected from the study.
4. Benefits to be expected from the study.
5. Alternative procedures, drugs or devices that might be helpful and their risks and benefits.
6. Availability of medical treatment should complications occur.
7. The opportunity to ask questions about the study or the procedure.
8. The opportunity to withdraw at any time without affecting your future care at this institution.
9. A copy of the written consent form for the study.
10. The opportunity to consent freely to the study without the use of coercion.
11. Statement regarding liability for research-related injury, if applicable.

Please initial each page:_____

AGREEMENT:

I have read (or someone has read to me) the information provided above. I have been given the opportunity to ask questions and all of my questions have been answered to my satisfaction. My signature below indicates that I have decided to participate having read the information provided above.

Name of Subject	Signature	Date Signed

Street Address	City	State, Zip

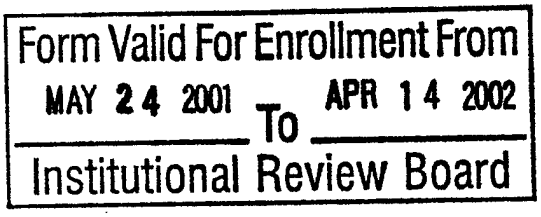
Thank you! Please mail the signed copy in the enclosed postage paid envelope or to the address listed below.

International Twin Study
Attn: Dr. Ann Hamilton
USC/Norris Comprehensive Cancer Center
1441 Eastlake Ave., Rm 3427, MC9175
Los Angeles, CA 90089-9175

Interviewer's statement and signature

'I have discussed the above information with the subject and answered all of the subject's questions regarding the study. It is my opinion that the subject understands the risks, benefits, and obligations involved in participation in this project'.

Name of Interviewer	Signature	Date Signed



USC Norris Comprehensive Cancer Center
Keck School of Medicine
Department of Preventive Medicine
1441 Eastlake Ave., Rm. 3427, MC9175
Los Angeles, CA 90089-9175

USC
SCHOOL OF
MEDICINE

3. Description of the Medical Research for which your participation is requested (Please sign and return one copy and keep the second copy for your records)

TITLE OF PROJECT: Breast Cancer Susceptibility Genes in High Risk Women

PRINCIPAL INVESTIGATOR: Ann Hamilton, Ph.D. (323)-865-0434

DEPARTMENT: Department of Preventive Medicine, Keck School of Medicine at the University of Southern California, 1441 Eastlake Ave, Los Angeles, CA 90089.

24-HOUR TELEPHONE NUMBER: Toll free number: 800-421-9631

PURPOSE OF THE STUDY: You are invited to participate in a research study of genetic factors that may be related to the development of breast cancer. These tests will be done in twin pairs with breast cancer in one or both members and in a group of women without breast cancer. You are invited to be a participant because you are a woman without breast cancer and have not had other family members with breast cancer.

The genes that we are studying include those that control the amount of estrogen produced in the body and those that control the removal of cancer causing substances from the body. (We will not be testing for BRCA1 and BRCA2, the rare genes that are known to increase risk of breast cancer). To test for these genes, we will use a sample of your DNA that will be obtained from a sample of cells from the inside of your mouth that would be collected by swabbing the inside of your mouth with a soft brush.

PROCEDURES: We obtained your name from a friend or relative of yours with your permission. We will send you a letter explaining this new study and ask you to collect a sample of cells from inside your mouth by swabbing the inside of your mouth with a soft brush and placing it in a plastic tube. You will be asked to mail the tube to us in a prepaid mailing envelope. In addition, you will be asked to complete a brief questionnaire on reproductive and lifestyle factors and mail it back to us in a prepaid mailing envelope. All of the materials and mailing supplies will be provided to you at no cost.

Laboratory analyses of the cell samples to determine the presence of genetic factors will be done with personal identifiers removed. Thus, it will not be possible to link results with specific individuals.

RISKS: There are no physical risks. For the sample collection in your mouth, there may be some slight discomfort. There is a small risk of loss of confidentiality with the completion of the questionnaire, however all records are secured and kept confidential. While genetic testing will be done for this study, the results cannot be linked to a specific individual, thus risks related to

genetic testing will not apply. For the genes being tested, the risk associated with development of breast cancer is unknown at this time.

BENEFITS: You may receive no direct benefit from your participation in this study. However, your participation may help us learn if certain genetic factors may be related to development of breast cancer.

ALTERNATIVES TO PARTICIPATION: An alternative would be not to participate in this study.

CONFIDENTIALITY STATEMENT: We will maintain the confidentiality of your medical records to the extent permitted by law. Data are kept in locked file cabinets and genetic information is not linked to personal identifying information. The information from this study may be published in scientific journals or presented at scientific meetings. Published results will only consist of numbers of persons arranged in categories. Representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as part of their responsibility to protect human subjects in research.

OFFER TO ANSWER QUESTIONS: You may contact the Principal Investigator at the number listed above if you have any questions about the study. If you have any questions regarding your rights as a research subject, you may contact the Institutional Review Board office (IRB) at 323-223-2340.

COMPENSATION: The availability and quality of your medical care will not be affected by your participation or refusal to participate. We can provide no compensation for your participation in this study.

VOLUNTARY PARTICIPATION AND WITHDRAWAL: Your participation in this research study is voluntary. Your decision whether or not to participate will not interfere with your right to health care or other services to which you are otherwise entitled. If you do decide to participate, you are free to withdraw your consent and discontinue participation at any time.

POTENTIAL FOR COMMERCIAL DEVELOPMENT RELATED TO RESEARCH AND DONATION OF CELL SAMPLES. The following paragraph is included at the request of the funding agency for the study, the Department of Defense. We feel that the possibility of a commercial application is remote.

During this study, you will be asked to provide cell samples from the inside of your mouth. These samples will be used for studying genes that may be related to breast cancer and may also be used for purposes that are currently unknown. There is a chance that the samples that you are donating under this study may be used in other research studies and may have some commercial value. Should your donated sample lead to the development of a commercial product, the Keck School of Medicine at the University of Southern California will own it and may take action to patent and license the product. The Keck School of Medicine at the University of Southern California does not intend to provide you with any compensation for your participation in this study nor for any future value that the sample you have given may be found to have.

	As a participant in this study called 'Breast Cancer Susceptibility Genes in High Risk Women', I voluntarily donate any and all cell samples to the Keck School of Medicine at the University of Southern California. I have read the above paragraph and agree that should my donated sample lead to the development of a commercial product, the Keck School of Medicine at the University of Southern California will own it and it is possible that it will be patented and licensed by the Keck School of Medicine at the University of Southern California . I will not receive any compensation for this.

Please write your initials in the box to indicate your agreement. Thank you.

INFORMATION ABOUT CELL SAMPLES COLLECTED AS PART OF THIS RESEARCH:

In the future it may be important to test these cells for newly discovered genetic factors that may be related to the development of breast cancer. We will be storing some of the cells for this purpose in the future. Please initial below if you give your consent for additional genetic testing to be done on these samples

	I give consent for additional genetic testing related to breast cancer to be done in the future on my stored cell samples.

Please write your initials in the box to indicate your agreement. Thank you.

CONSENT PROCEDURE: Initial contact with participants in this study is made by a letter describing the study purpose and procedures. Participants are called and this document is discussed with them and they are asked to sign and return a copy of this document.

CALIFORNIA LAW REQUIRES THAT YOU MUST BE INFORMED ABOUT:

1. The nature and purpose of the study.
2. The procedures in the study and any drug or device to be used.
3. Discomforts and risks to be expected from the study.
4. Benefits to be expected from the study.
5. Alternative procedures, drugs or devices that might be helpful and their risks and benefits.
6. Availability of medical treatment should complications occur.
7. The opportunity to ask questions about the study or the procedure.
8. The opportunity to withdraw at any time without affecting your future care at this institution.
9. A copy of the written consent form for the study.
10. The opportunity to consent freely to the study without the use of coercion.
11. Statement regarding liability for research-related injury, if applicable.

AGREEMENT:

I have read (or someone has read to me) the information provided above. I have been given the opportunity to ask questions and all of my questions have been answered to my satisfaction. My signature below indicates that I have decided to participate having read the information provided above.

Name of Subject	Signature	Date Signed

Street Address	City	State, Zip

Thank you! Please mail the signed copy in the enclosed postage paid envelope or to the address listed below.

International Twin Study
Attn: Dr. Ann Hamilton
USC/Norris Comprehensive Cancer Center
1441 Eastlake Ave., Rm 3427, MC9175
Los Angeles, CA 90089-9175

Interviewer's statement and signature

'I have discussed the above information with the subject and answered all of the subject's questions regarding the study. It is my opinion that the subject understands the risks, benefits, and obligations involved in participation in this project'.

Name of Interviewer	Signature	Date Signed